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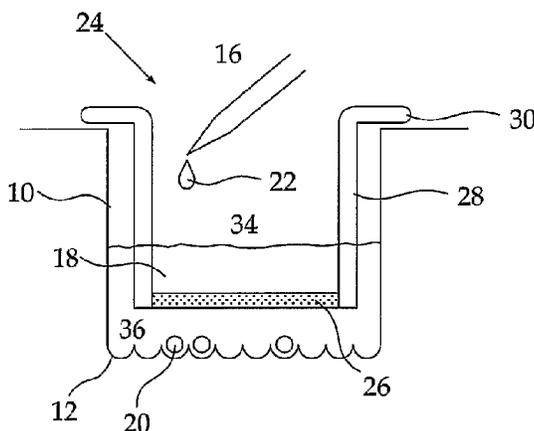
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(54) **Title:** CURRENT DAMPER FOR THE STUDY OF CELLS



(57) **Abstract:** A device for the study of cells (20) including a vessel (10) with a current damper (24) including a damping component substantially disposed within the vessel is disclosed. The damping component reduces or eliminates currents formed by the addition of materials such as liquids to the vessel to prevent the movement of cells resting on the bottom surface of the vessel.

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## CURJRENT DAMPER FOR THE STUDY OF CELLS

5 FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to the field of scientific devices and more particularly, to an improved device for the study of cells. Specifically, the present invention is of a device allowing the addition of a material to a vessel without disturbing cells held in the vessel.

10 Combinatorial methods in chemistry, cellular biology and biochemistry are essential for the near simultaneous preparation of multitudes of active entities such as molecules. Once such a multitude of molecules is prepared, it is necessary to study the effect of each one of the active entities on a living organism.

The study of the effects of stimuli such as exposure to active entities on living  
15 organisms is preferably initially performed on living cells. Since cell-functions include many interrelated pathways, cycles and chemical reactions, the study of an aggregate of cells, whether a homogenous or a heterogeneous aggregate, does not provide sufficiently detailed or interpretable results; rather a comprehensive study of the biological activity of an active entity may be advantageously performed by examining  
20 the effect of the active entity on a single isolated living cells. Thus, the use of single-cell assays is one of the most important tools for understanding biological systems and the influence thereupon of various stimuli such as exposure to active entities.

The combinatorial preparation of a multitudes of active entities coupled with the necessity of studying the effect of each one of the active entities on living organisms  
25 using single-cell assays, requires the development of high-throughput single live cell assays. There is a need for the study of real-time responses to stimuli in large and heterogeneous cell populations at an individual cell level. In such studies it is essential to have the ability to define multiple characteristics of each individual cell, as well as the individual cell response to the experimental stimulus of interest.

30 In the art, various methods and devices for studying living cells are known.

One method of studying cells involves placing cells on a bottom surface of a vessel and observing the behavior of the cell in response to stimuli. Typically used vessels include slides with recesses and Petri dishes. To allow for simultaneous study of

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distinct groups of cells exposed to similar or different stimuli, multiwell plates are most commonly used. Multiwell plates are substantially a group of individual vessels of a standard size physically associated in a standard way allowing for simplified simultaneous or sequential studies of different groups of cells. Multiwell plates having  
5 6, 12, 24, 48, 96, 384 or even 1536 wells on a standard *ca.* 8.5 cm by *ca.* 12.5 cm footprint are well known in the art. Such multiwell plates are provided with an  $2n$  by  $3n$  array of rectangular packed wells,  $n$  being an integer. The diameter of the wells of a plate depends on the number of wells and is generally greater than about 250 microns (for a 1536 well plate). The volume of the wells depends on the number of wells and the  
10 depth thereof but generally is greater than  $5 \times 10^{-6}$  liter (for a 1536 well plate). The standardization of the multiwell plate format is a great advantage for researchers, allowing the use of standardized products including robotic handling devices, automated sample handlers, sample dispensers, plate readers, observation components, plate washers, software and such accessories as multifilters.

15 When a vessel having a planar bottom surface is used to study cells, the cells are most often studied as a group having an aggregate of properties of the individual cells. Since the cells are studied as a group, in such studies the identity of individual cells is not important. Such studies are of limited utility due to the fact that naturally occurring cell populations are rarely homogenous and often it is the heterogeneity and the  
20 differences of behavior of cells that is interesting.

Efforts have been made to use vessels having a planar bottom surface to study cells as individuals but such efforts are plagued with many difficulties. A first difficulty is that cells have a tendency to clump together in variably sized groups at random locations, and often stack one on top of the other. The clumping and stacking of cells  
25 together makes it virtually impossible to delineate the borders of one cell from another, see discussion in unpublished PCT Patent Application No. IL2005/000719 of the inventor. It is thus virtually impossible to identify which cell has a given behavior. Further, the fact that cells are randomly distributed over a featureless surface makes it impossible to definitely differentiate one cell from another without continuous  
30 observation of the cell.

The greatest difficulty limiting the utility of such methods is that even the slightest current, whether caused by addition of a material to the vessel or by movement,

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*e.g.* incidental jostling, of the vessel causes the cells to move randomly leading to the loss of identity of the cells and rendering experiments difficult to perform, limited in scope and slow. In Figure IA, a microwell 10 defined by a bottom surface 12 and vessel walls 14 is schematically depicted in cross section. A plurality of cells 20 rest at various  
5 locations on bottom surface 12. Cell density is relatively low to reduce as much as possible the clumping of cells 20. When a material 22 is added from surroundings 16, material 22 enters vessel 10 and mixes with liquid 18 held in vessel 10. When material 22 is added currents formed by the impact of material 22 with liquid 18 are sufficient to cause cells 20 to shift from a given location.

10 It is known to provided vessels having cell-localizing features arranged in arrays on a planar surface. Cells are held in a specific location that is individually addressable allowing the identity of a given cell to be retained even without continuous observation. Many such devices bind or adhere to the surface of the cells or deform the shape of the cells, adversely effecting the results of performed studies, see for example Mrksich and  
15 Whitesides, *Ann. Rev. Biophys. Biomol. Struct.* 1996, 25, 55-78; Craighead *et al*, *J. Vac. Sci Technol.* 1982, 20, 316; Singhvi *et al*, *Science* 1994, 264, 696-698; Aplin and Hughes, *Analyt. Biochem.* 1981, 113, 144-148, U.S. Patent No. 4,729,949, U.S. Patent No. 5,324,591, U.S. Patent No. 6,103,479 and PCT Patent Application No. US99/04473 published as WO 99/45357.

20 In PCT patent applications IL2001/00992 published as WO2003/035824, IL2004/000571 published as WO2004/1 13492 and IL2004/000194 published as WO2004/077009, all of the inventor, are provided devices provided with a plurality of picowells for the study of cells. In such devices, individual cells are held unadhered and in a substantially natural state in individual adressable picowells. The term "picowell" is  
25 general and includes such features as dimples, depressions, tubes and enclosures. Since cells range in size from about 1 microns to about 100 (or even more) microns diameter there is no single picowell size that is appropriate for holding a single cell of any type. That said, the dimensions of the typical individual picowell in the picowell-bearing components known in the art have dimensions of between about 1 microns up to about  
30 200 microns, depending on the exact implementation. Using such devices large number of cells are studied as individuals. Complex experiments involving serial addition of reagents and the like are performed with dedicated microfluidics. Despite the

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unparalleled utility of the devices taught in PCT patent applications IL2001/00992 published as WO2003/035824, IL2004/000571 published as WO2004/113492 and IL2004/000194 published as WO2004/077009, such devices have a number of disadvantages. A first disadvantage is the need for flow generators and concomitant  
5 interfaces that increases the complexity of such devices. A second disadvantage is that the difficulties in the use of the device including loading, attaching flow generators and the like render the integration of such a device with a robotics system for automatised use impractical.

Problems associated with the need for flow generators and complex interfaces  
10 are overcome in unpublished PCT patent application IL2005/000801 of the inventor where a device including an array of picowells that is configured for easy coupling to a robotics system for automatised use is taught. Although extremely useful, the device taught in PCT patent application IL2005/000801 does not provide a general solution allowing the use of existing vessels for the study of cells.

15 In PCT patent application IL2004/000661 published as WO2005/007796 of the inventor is taught a well-bearing device, where on the bottom surface of each well is a plurality of picowells. A preferred embodiment of the device is substantially a standard 96-well plate where the bottom of each well is covered with a plurality of picowells. Such a device allows the use of standard robotics and other standard accessories to  
20 study cells, while the picowells allows a high density of cells to be held in a microwell without cell clumping and where each cell is addressable. Unfortunately under certain conditions currents in a liquid held in a well may cause cells held in picowells to move and thus lose identity. In Figure IB a microwell 10 defined by a bottom surface 12 and vessel walls 14 of a device of PCT patent application IL2004/000661 is schematically  
25 depicted in cross section. As is seen, bottom surface 12 is entirely covered with picowells, each picowell configured to hold one cell 20 separated from other cells 20. When a material 22 is added from surroundings 16, material 22 enters vessel 10 and mixes with liquid 18 held in vessel 10. It has been found that when a relatively large amount of material 22 is added, or material 22 is added at a high velocity (for example  
30 when ejected from a pipette or an automatic injector such as used in robotic devices), currents formed by the impact of material 22 with liquid 18 may cause cells 20 to move from one picowell to another leading to the loss of the identity of cells 20.

It would be highly advantageous to have a device for the study of cells not having at least some of the disadvantages of the prior art.

#### SUMMARY OF THE INVENTION

5           The present invention successfully addresses at least some of the shortcomings of the prior art by providing a damping component disposed within a cell-holding vessel, such as a microwell. When material (*e.g.*, a fluid or solid) is added to the vessel or when a device (*e.g.*, a probe or a detector) is placed in the vessel the damping component damps, that is to say, prevents or reduces currents, turbulence or flows that  
10 otherwise would cause cells held in the vessel, and especially resting on the bottom of the vessel, to move.

Thus, according to the teachings of the present invention there is provided a device for the study of cells comprising: (a) a vessel including a bottom surface and a wall having a bottom edge, a top edge defining a rim of the vessel, the rim surrounding  
15 an opening; and (b) a current damper including a damping component substantially disposed within the vessel.

In embodiments of the present invention, the damping component substantially divides the vessel into at least two volumes, a first volume including at least part of the bottom surface and a second volume in fluid communication with the surroundings and  
20 with the first volume.

In embodiments of the present invention the current damper is fixedly associated with the vessel. In embodiments of the present invention the current damper is discrete from the vessel. In embodiments of the present invention the damping component is discrete from the vessel.

25           In embodiments of the present invention the damping component comprises a damping surface. In embodiments of the present invention the damping surface is planar and is preferably substantially parallel to the bottom surface. In embodiments of the present invention the damping surface is not-planar, (*e.g.*, curved) or not parallel to the bottom surface.

30           In embodiments of the present invention the damping surface is porous. In embodiments of the present invention the damping surface is permeable to liquids. In embodiments of the present invention the damping surface is a net (that is, having a net-

like shape or form) having relatively large spaces for fluids to pass therethrough. In embodiments of the present invention the damping surface is a porous membrane, especially a microporous membrane.

In embodiments of the present invention where the damping component  
5 substantially divides the vessel into two volumes, a first volume including at least part of the bottom surface and a second volume in fluid communication with the surroundings and with the first volume, preferably fluid communication between the surroundings and the first volume is substantially only via the second volume. In  
10 embodiments of the present invention the fluid communication between the first volume and the second volume is substantially only through a damping surface.

In embodiments of the present invention the damping component is configured to optionally allow substantially unimpeded fluid communication between the first volume and the surroundings. In embodiments of the present invention the substantially unimpeded fluid communication is through a gap in the damping surface. In  
15 embodiments of the present invention the substantially unimpeded fluid communication is past a side of the damping surface.

In embodiments of the present invention the current damper is configured to allow at least part of the damping component to rest on the surface of a liquid contained in the vessel. In embodiments of the present invention the damping component is  
20 configured to float on the surface of a liquid (especially an aqueous liquid) contained in the vessel.

In embodiments of the present invention the damping surface is configured to be entirely submerged in a liquid held in the vessel. In such an embodiment a current damper is more effective in also damping currents cause by movement of the vessel.

In embodiments of the present invention the vessel is substantially parallel-walled and the damping component has an outer edge having dimensions substantially similar to dimensions of a cross-section defined by the parallel walls.

25 In embodiments of the present invention the damping component is configured to maintain the damping surface at a substantially fixed distance above the bottom surface of the vessel. In embodiments of the present invention the fixed distance is no more than about 5 mm, no more than about 3 mm, no more than about 1.5 mm, no more than about 1 mm, no more than about 500 microns, no more than about 300 microns, no

more than about 200 microns, no more than about 100 microns and even no more than about 50 microns

In embodiments of the present invention the current damper further comprises a retaining component configured to physically engage the top edge of the vessel. In  
5 embodiments of the present invention the retaining component and the damping component are fixedly associated. In embodiments of the present invention the retaining component and the damping component are moveably associated.

In embodiments of the present invention the current damper further comprises a retaining component configured to rest on the bottom surface of the vessel.

10 In embodiments of the present invention the retaining component and the damping component are fixedly associated. In embodiments of the present invention the retaining component and the damping component are reversibly associated.

In embodiments of the present invention the current damper comprises a plurality of damping components, each damping component configured to be disposed  
15 with a separate vessel. In embodiments of the present invention the plurality is selected from the group consisting of 6, 12, 24, 48, 96, 384 and 1536.

According to the teachings of the present invention there is provided a method for the study of cells comprising (a) providing a vessel including a bottom surface and a wall having a bottom edge, a top edge defining a rim of the vessel, the rim surrounding  
20 an opening, (b) placing at least one cell in a liquid (*i.e.*, a cell solution) in the vessel at a location on the bottom surface, (c) disposing a damping component of a current damper within the vessel, and (d) adding a material (*e.g.*, a liquid or a solid) to the vessel wherein the damping component damps currents thereby reducing movement of the at least one cell from the location as a result of the adding of the material.

25 In embodiments of the present invention, the damping component substantially divides the vessel into at least two volumes, a first volume including at least part of the bottom surface and a second volume in fluid communication with the surroundings and with the first volume. In embodiments of the present invention, the at least one cell is placed in the first volume. In embodiments of the present invention, adding the material  
30 is into the second volume. In embodiments, subsequent to the adding of the material into the second volume, the material passes into the first volume from the second volume.

In embodiments of the present invention the cell solution is placed on the bottom surface before the damping component is disposed within the vessel. In embodiments of the present invention the at least one cell is placed in the vessel on the bottom surface after the damping component is disposed within the vessel.

5           Disposing the damping component in the vessel includes during manufacture, for example integrally forming the damping component with the vessel or attaching during manufacture, and also includes laying a discrete damping component inside of the well before or after the cell solution is added to the vessel.

10           In embodiments of the present invention, the damping component comprises a damping surface and adding the material includes contacting the material with the damping surface.

In embodiments of the present invention, the damping surface is not planar.

15           In embodiments of the present invention, the damping surface is substantially unparallel to the bottom surface. In embodiments of the present invention, the damping surface is substantially perpendicular to the bottom surface.

In embodiments of the present invention, the damping surface is substantially entirely submerged in the liquid. The advantage of such an embodiment is that the damping component is then more effective at damping currents caused by movement of the vessel.

In embodiments of the present invention the damping surface is substantially planar. In embodiments of the present invention the damping surface is substantially parallel to the bottom surface of the vessel. In embodiments of the present invention the damping surface is substantially at a fixed distance from the bottom surface. In embodiments of the present invention the fixed distance is no more than about 5 mm, no more than about 3 mm, no more than about 1.5 mm, no more than about 1 mm, no more than about 500 microns, no more than about 300 microns, no more than about 200 microns, no more than about 100 microns and even no more than about 50 microns.

In embodiments of the present invention, the damping surface is porous and/or permeable and subsequent to adding the material, the material passes through the damping surface.

20           In embodiments of the present invention, at least part of the damping component (*e.g.*, the damping surface) rests (*e.g.*, floats) on the surface of the liquid in the well.

In embodiments of the present invention at least part of the damping component rests on the bottom surface of the vessel.

In embodiments of the present invention the damping component is suspended within the vessel above the bottom surface.

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#### BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of  
10 illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the  
15 description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1A (prior art) schematically depicts addition of a material to a microwell of  
20 a 96-well plate;

FIG. 1B (prior art) schematically depicts addition of a material to a microwell of a 96-well plate provided with picowells as taught in PCT patent application No. IL2004/000661 published as WO2005/007796;

FIG. 2A depicts an embodiment of a current damper of the present invention;

25 FIG. 2B schematically depicts addition of a material to a microwell in which the current damper depicted in Figure 2A is disposed;

FIG. 2C schematically depicts removal of a liquid from a microwell in which the current damper depicted in Figure 2A is disposed;

30 FIG. 3 depicts an alternative embodiment of a current damper of the present invention;

FIG. 4 depicts an alternative embodiment of a current damper of the present invention;

FIGS. 5A and 5B depict an alternative embodiment of a current damper of the present invention;

FIG. 6 depicts an alternative embodiment of a current damper of the present invention;

5 FIG. 7 depicts an alternative embodiment of a current damper of the present invention;

FIG. 8 depicts an alternative embodiment of a current damper of the present invention;

10 FIG. 9 depicts an alternative embodiment of a current damper of the present invention; and

FIG. 10 depicts an alternative embodiment of a current damper of the present invention.

15 In the figures herein features such as cells 20 are depicted out of scale for illustrative purposes.

#### EMBODIMENTS OF THE INVENTION

20 The present invention is of a device for the study of cells comprising a vessel for holding cells, where the cells rest on the bottom surface of the vessel, and a current damper including a damping component substantially disposed within the vessel. The present invention is also of a current damper configured to be placed in, or disposed within, a vessel. The present invention is also of a method for using a device of the present invention. While a cell or cells are resting on the bottom surface of the vessel a material (*e.g.*, a liquid or solid reagent) is added to the vessel or a device (*e.g.*, a probe  
25 or a detector) is placed in the vessel. The damping component preferably damps currents caused by the addition of the material or the placement of the device that would otherwise cause movement of the cell or cells. The damping component preferably also damps currents caused by movement of the vessel that would otherwise cause movement of the cell or cells.

30 The principles and uses of the teachings of the present invention may be better understood with reference to the accompanying description, figures and example. In the figures, like reference numerals refer to like parts throughout.

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Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth herein. The invention can be implemented with other embodiments and can be practiced or carried out in various ways. It is also understood that the phraseology and terminology employed herein is for descriptive purpose and should not be regarded as limiting.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include techniques from the fields of biology, chemistry, engineering and physics. Such techniques are thoroughly explained in the literature.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. In addition, the descriptions, materials, methods, and examples are illustrative only and not intended to be limiting. Methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention.

As used herein, the terms "comprising" and "including" or grammatical variants thereof are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof. This term encompasses the terms "consisting of" and "consisting essentially of."

The phrase "consisting essentially of" or grammatical variants thereof when used herein are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof but only if the additional features, integers, steps, components or groups thereof do not materially alter the basic and novel characteristics of the claimed composition, device or method.

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts. Implementation of the methods of the present

invention involves performing or completing selected tasks or steps manually, automatically, or a combination thereof.

Herein, the term "active entity" is understood to include chemical, biological or pharmaceutical entities including any natural or synthetic chemical or biological substance that influences a cell with which the entity is in contact. Typical active entities include but are not limited to active pharmaceutical ingredients, antibodies, antigens, biological materials, chemical materials, chromatogenic compounds, drugs, enzymes, fluorescent probes, immunogenes, indicators, ligands, nucleic acids, nutrients, peptides, physiological media, proteins, receptors, selective toxins and toxins.

Herein, by "indicator" is meant any active entity that upon interaction with some stimulus produces an observable effect. In the context of the present invention, by stimulus is meant, for example, a specific second active entity (such as a molecule) released by a cell and by observable effect is meant, for example, a visible effect, for example a change in color or emission of light.

Herein, by "picowell array" is meant a group of one or more picowells, preferably a plurality of picowells, preferably a plurality of picowells arranged in an orderly fashion.

Some embodiments of the present invention include components that are transparent or are made of a transparent material. By "transparent" is meant that the component or material is substantially transparent to at least one wavelength of light (preferably a range of wavelengths) in at least part of the visible light spectrum, the ultraviolet light spectrum and/or of infrared radiation, preferably the visible light spectrum.

Generally, the present invention is based on disposing a damping component in a vessel holding a liquid and having a bottom surface on which small particles such as a cell or cells rest. Generally, the damping component divides the vessel into at least two volumes. The first volume includes at least part of the bottom surface and therefore the cells and at least some liquid while the second volume is in fluid communication with the first volume through the damping component.

When a material is added directly into the first volume, for example by using a pipette, the speed and mass of the added material produces currents in the liquids that

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causes the cell or cells to move, causing a loss of identity of the cells as discussed in the introduction.

When material is added into the second volume currents are produced in the second volume. However the currents are damped by the current damper and do not  
5 substantially pass into the first volume. Rather, the added material passes through the damping component to contact the cells with little, no or substantially no disturbing of the cells.

Thus, the teachings of the present invention allows the addition of a material such as a liquid, reagent or active entity to a vessel where cells are resting on the bottom  
10 surface of the vessel without fear that the addition of the the material will cause currents that will move the cells.

In embodiments of the present invention, addition of materials to the first (cell-containing) volume from the surroundings is substantially only through the damping component via the second volume.

15 In embodiments of the present invention, the device is configured to allow optional bypassing of the damping component to allow addition of material to the first volume from the surroundings.

In Figure 2A, a current damper 24 of the present invention is depicted in perspective. Current damper 24 includes a damping surface 26, a circular wall 28 and a  
20 flange 30 provided with a pressure equalizing notch 32.

Damping surface 26 is a substantially planar circular piece of 3 micron microporous polycarbonate filter membrane attached to the bottom rim of circular wall 28. Although damping surface 26 depicted in Figure 2A is a microporous membrane, non-depicted embodiments of permeable damping surfaces of the present invention  
25 exist, for example where a damping surface is substantially a net or net-like arrangement of fibers or the like or an otherwise permeable membrane.

Circular wall 28 is configured to be disposed within an appropriate vessel, for example a microwell 10 of a standard 96-well plate or of a 96-well plate provided with picowells as described in PCT patent application IL2004/000661 published as  
30 WO2005/007796 of the Applicant, as depicted in Figures 2B and 2C.

Flange 30 is an integrally molded part of circular wall 28, flaring from the top rim of circular wall 28. Flange 30 constitutes a retaining component of current damper

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24 and is configured to engage the top surface in the proximity of the rim of a microwell 10 to suspend damping surface 26 substantially in parallel to and at a distance of 200 microns above bottom surface 12. Generally the preferred distance that a damping surface is suspended above a bottom surface of a vessel is dependent on many factors such as vessel size or vessel volume and is determined for a specific implementation. That said, in embodiments of the present invention a damping surface is suspended no more than about 5 mm, no more than about 3 mm, no more than about 1.5 mm, no more than about 1 mm, no more than about 500 microns, no more than about 300 microns, no more than about 200 microns, no more than about 100 microns and even no more than about 50 microns above the bottom surface of a respective vessel.

The use of current damper 24 is schematically depicted in Figures 2B and 2C.

A physiological solution 18 including cells 20 is placed inside microwell 10. Cells 20 are allowed to settle into picowells on bottom surface 12. The damping component of current damper 24 consisting of circular wall 28 and damping surface 26 is placed inside microwell 10 so that flange 30 (constituting the retaining component of current damper 24) engages the top surface in the proximity of the rim of a microwell 10 as discussed above. As current damper 24 settles into microwell 10, trapped air escapes through pressure equalizing notch 32.

As is seen in Figures 2B and 2C, when current damper 24 is in place, vessel 10 is divided into two volumes: a second volume 34 substantially above damping surface 26 and a first volume 36 substantially between damping surface 26 and bottom surface 12. First volume 36 is in fluid communication with surroundings 16 substantially only through damping surface 26 via second volume 34. Damping surface 26 is entirely submerged in liquid 18 held in vessel 10. A substantially submerged or an entirely submerged damping surface such as 26 depicted in Figures 2B and 2C is more effective than other configurations in damping of currents caused by movement of a respective vessel such as 10 and is thus preferred.

In Figure 2B, addition of a material 22 to microwell 10 provided with current damper 24 is depicted. Material 22 placed in second volume 34 makes contact with and passes through damping surface 26 into first volume 36 and particularly to bottom surface 12 and cells 20. The addition of material 22 to second volume 34 causes currents, but as passage of material 22 is through damping surface 26, currents caused

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by the addition of material 22 to microwell 10 are substantially isolated to second volume 34 and damped in first volume 36 so cells 20 are only a little, not or substantially not disturbed and stay substantially in location on bottom surface 12.

5 In Figure 2C, removal of liquid 18 from microwell 10 provided with current damper 24 is depicted. Liquid 18 is removed from second volume 34, lowering the level of liquid 18 in second volume 34. Subsequently, liquid 18 in first volume 36 passes up through damping surface 26 into second volume 34 to equalize the respective liquid level. As the passage of liquid 18 is through damping surface 26, currents caused by removal of liquid 18 from microwell 10 are substantially isolated to second volume 34  
10 and damped in first volume 36 so cells 20 are only a little, not or substantially not disturbed and stay substantially in location on bottom surface 12. It is important to note that current damper 24 is fashioned to be sufficiently heavy or is attached to microwell 10 in order to avoid buoyancy-induced shifting when liquid 18 is removed from microwell 10.

15 In Figure 3 is depicted a current damper 38 of the present invention in perspective with a portion cut out, disposed inside a vessel 10, depicted in phantom. Current damper 38 is similar to current damper 24 depicted in Figures 2A-2C with a significant difference that current damper 38 is provided with tubular internal wall 40 rigidly attached to circular wall 28 through ribs 42. As in current damper 24 depicted in  
20 Figures 2A-2C, current damper 38 is provided with a substantially planar porous damping surface 26.

When current damper 38 is disposed within a vessel 10, flange 30 engages the top surface in the proximity of the rim of vessel 10 so that damping surface 26 is suspended substantially in parallel to and at a fixed distance above bottom surface 12 of  
25 vessel 10. Further, when current damper 38 is in place, vessel 10 is divided into two volumes: a second toroidal volume 34 substantially above damping surface 26 and a first volume 36 substantially between damping surface 26 and bottom surface 12. First volume 36 is in fluid communication with surroundings 16 through damping surface 26 via second volume 34 and also directly and unimpeded through circular inner wall 40.

30 Damping component 38 is optionally used substantially as described above for damping component 24. The addition of a material 22 to second volume 34 causes currents, but as passage of material 22 is through damping surface 26, currents caused

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by the addition of material 22 to microwell 10 are substantially isolated to second volume 34 and damped in first volume 36 so cells 20 are only a little, not or substantially not disturbed and substantially stay in location on bottom surface 12. However, first volume 36 can also be accessed directly and unimpeded through upper opening 44 of circular inner wall 40. An advantage of direct and unimpeded access is that damping component 38 can be disposed in vessel 10 and subsequently a solution including cells 20 added through upper opening 44 directly into first volume 36 to settle onto bottom surface 12 of vessel 10.

In Figure 4 is depicted a current damper 46 of the present invention in perspective in place inside a vessel 10, depicted in phantom. Current damper 46 includes a lower ring 48 and an upper ring 50 conjoined by ribs 52 to define a truncated conical skeleton to which a non-planar microporous membrane damping surface 26 is attached.

For use, current damper 46 is placed inside an appropriate vessel 10 with lower ring 48, constituting a retaining component, resting on bottom surface 12 of vessel 10. Lower ring 48 is configured to contact vessel walls 14 of vessel 10 in proximity of bottom surface 12 to hold current damper 46 snugly in place. When current damper 46 is in place in vessel 10, vessel 10 is divided into two volumes: a second volume 34 substantially above damping surface 26 and a second volume substantially between damping surface 26 and bottom surface 12 of vessel 10. First volume 36 is in fluid communication with surroundings 16 through damping surface 26 via second volume 34 and also directly and unimpeded through the bore of upper ring 50.

Analogously to current damper 38 depicted in Figure 3, current damper 46 allows addition and removal of materials from first volume 36 either directly and unimpeded or through damping surface 26 to dampen potentially produced currents.

A current damper 54 of the present invention is depicted in a disassembled state in Figure 5A (side view and perspective) and in Figure 5B in an assembled state (in perspective) as part of vessel 10 (depicted in phantom). Unlike the previously depicted embodiments of the device of the present invention, components of current damper 54 are fixedly associated with vessel 10. Current damper 54 includes a tubular microporous membrane damping surface 26 that is removeably held in place by support pegs 56 that are integrally formed with bottom surface 12 of vessel 10 and function as retaining

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components. Damping surface 26 is placed over support pegs 56 for assembly of current damper 54 so that vessel 10 is divided into two volumes: second volume 34 substantially the tubular volume between vessel walls 14 and damping surface 26 and first volume 36 substantially the cylindrical volume enclosed by damping surface 26.

5 For use, a solution containing cells is placed in first volume 36 so that the cells settle on bottom surface 12 inside first volume 36. Surroundings 16 are in fluid communication with first volume 36 both through damping surface 26 via second volume 34 or directly and unimpeded from the area of the opening of vessel 10 delineated by the top of damping surface 26. Analogously to current damper 38 depicted  
10 in Figure 3, current damper 54 allows addition and removal of materials from first volume 36 either directly and unimpeded or through damping surface 26 to dampen potentially produced currents.

A current damper 58 of the present invention is depicted in Figure 6 disposed within vessel 10 depicted in phantom. Current damper 58 is fixedly associated with  
15 vessel 10 using adhesive. Unlike previously depicted embodiments of the present invention, current damper 58 does not include a damping surface. Current damper 58 is substantially a bent tube 60 having a relatively large diameter at an inlet 62 and a relatively small diameter at an outlet 64 substantially constituting a self-contained vessel and defining a second volume 34. The part of vessel 10 not taken up by current  
20 damper 58 constitutes a first volume 36. When a liquid material is introduced through inlet 62 into second volume 34, the material passes through bent tube 60 and emerges out into first volume 36 through outlet 64. The reduction of the diameter of bent tube 60 from inlet 62 to outlet 64 reduces the rate of entry of the material to first volume 36. Further, material exiting through outlet 64 flows along the outer wall of bent tube 60 in  
25 proximity of outlet 64. Both the reduced exit flow rate and the flow along the outer wall of bent tube 60 damps currents potentially produced by the addition of the material. For use, a solution containing cells is placed in first volume 36 so that the cells settle on bottom surface 12 inside first volume 36. Surroundings 16 are in fluid communication with first volume 36 both through second volume 34 via outlet 64 or directly and  
30 unimpeded through the top opening of vessel 10. Analogously to current damper 38 depicted in Figure 3, current damper 58 allows addition of materials to first volume 36

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either directly and unimpeded or through second volume 34 to dampen potentially produced currents.

A current damper 66 of the present invention is depicted in Figure 7 disposed within vessel 10 depicted in phantom. Current damper 66 includes a frame 70 to which  
5 a planar microporous membrane damping surface 26 is attached. Frame 70 is fixedly attached by welding to bottom surface 12 and at two sides of vessel wall 14. In such a way, vessel 10 is divided into two volumes in fluid communication through damping surface 26: a second volume 34 substantially above damping surface 26 and a first volume 36 substantially between damping surface 26 and bottom surface 12 of vessel  
10 10. First volume 36 is in fluid communication with surroundings 16 through damping surface 26 via second volume 34 and also directly and unimpeded through upper opening of vessel 10. Analogously to current damper 38 depicted in Figure 3, current damper 66 allows addition and removal of materials from first volume 36 either directly and unimpeded or through damping surface 26 to dampen potentially produced currents.

15 A current damper 70 of the present invention is depicted in Figure 8 disposed within vessel 10 depicted in phantom. Current damper 70 includes a circular frame 72 to which a planar microporous membrane damping surface 26 is attached. Current damper 70 is a discrete component configured to float on the surface of a liquid held in vessel 10 continuously maintaining damping surface 26 parallel to bottom surface 12 at a  
20 distance that varies with the depth of the liquid held in vessel 10. Circular frame 72 has an outer edge having dimensions substantially similar to those of a cross-section of vessel 10 so that circular frame 72 moves freely up and down inside vessel 10 with little space between circular frame 72 and vessel wall 14. In such a way, vessel 10 is divided into two volumes in fluid communication substantially exclusively through damping surface 26: a second volume 34 above damping surface 26 and a first volume 36 below  
25 damping surface 26. Surroundings 16 are in fluid communication with first volume 36 only through damping surface 26 via second volume 34. Analogously to current damper 24 depicted in Figures 2A-2C, current damper 70 damps currents potentially produced in first volume 36 when materials are added to second volume 34.

30 A current damper 74 of the present invention is depicted in Figure 9 disposed within vessel 10 depicted in phantom. Current damper 74 includes a frame 76 to which a planar microporous membrane damping surface 26 is attached. Current damper 74 is a

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discrete component configured to rest on bottom surface 12 of vessel 10 on integrally formed feet 78 which constitute retaining components of current damper 74. Feet 78 and frame 82 are configured to maintain damping surface 26 parallel with and at a fixed distance from bottom surface 12. Frame 76 is configured to snugly fit inside vessel 10 and to contact vessel walls 14 except in the vicinity of upwardly extending inwardly arcing solid wall 80. In such a way, vessel 10 is divided into two volumes: a second volume 34 above damping surface 26 and a first volume 36 below damping surface 26. Second volume 34 and first volume 36 are in fluid communication through damping surface 26. Further, as solid wall 80 arcs inwardly away from vessel wall 14, solid wall 80 defines a passage 82. Passage 82 allows direct and unimpeded fluid communication of first volume 36 with surroundings 16. Analogously to current damper 38 depicted in Figure 3, current damper 74 allows addition of materials to first volume 36 either directly and unimpeded through passage 82 past the side of damping surface 26 or through damping surface 26 to dampen potentially produced currents.

In Figure 10 is depicted a current damper 84 of the present invention. Current damper 84 is substantially planar and includes 96 individual damping components 86 arranged in an 8 by 12 array suitable for coupling with a standard 96 well plate. Each individual damping component 86 is substantially similar to current damper 38 depicted in Figure 3 with the exception that a plate 88 acts as a retaining component instead of a flange 30 of current damper 34. The size of each individual damping component 86 as well as the spacing and arrangement of the array is such that when current damper 84 is coupled with a standard 96 well plate, a damping component 86 is disposed inside each one of the 96 individual microwells. Whereas current damper 84 is provided with 96 individual damping components 86 to allow coupling with a standard 96 well plate, preferred non-depicted embodiments of the present invention are provided with 6, 12, 24, 48, 384 or 1536 individual damping components of the appropriate size and arranged in an array to allow coupling with standard format plates having 6, 12, 24, 48, 384 or 1536 microwells.

### 30 *Methods of manufacture of a current damper of the present invention*

In general, manufacture and assembly of a current damper of the present invention is well within the ability of one skilled in the art upon perusal of the

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description and figures herein using any suitable method with which one skilled in the art is well acquainted. Suitable methods include methods that employ one or more techniques including but not limited to casting, embossing, etching, free-form manufacture, injection-molding, microetching, micromachining, microplating, molding, spin coating, lithography or photo-lithography.

Suitable materials from which to manufacture components of a current damper include but are not limited to ceramics, epoxies, glasses, glass-ceramics, metals, plastics, polycarbonates, polydimethylsiloxane, polyethyleneterephthalate glycol, polymers, polymethyl methacrylate, paraffins, polystyrene, polytetrafluoroethylene, polyurethanes, polyvinyl chloride, silicon, silicon oxide silicon rubbers.

In embodiments of the present invention, a current damper includes a component made of a permeable membrane such as a microporous membrane. Suitable membranes for use as components of a device of the present invention include but are not limited to collagen, polyethersulfone, nitrocellulose mixed esters, polyamide (Nylon), polycarbonate, polyester, polyvinylidene fluoride, cellulose acetate and polytetrafluoroethylene. Suitable membranes are commercially available, for example, Magna™ membranes from Osmonics, Inc., Minnetonka, MN).

Membrane components of a current damper of the present invention are attached to other components of the current damper with the usual methods, for example using adhesives, welding or clamping of the membrane between two other components.

In a preferred embodiment, a damping component of the present invention is made of, at least in part, of a transparent material having an index of refraction that is close to that of water (*e.g.*, less than about 1.4, less than about 1.38, or even less than 1.35), for example made of polytetrafluoroethylene. When such a material is used that part is effectively invisible, avoiding optical study of cells held in the vessel where the damping component is disposed. For example, when a damping surface of a damping component such as depicted in Figure 2A, depicted in Figure 7, depicted in Figure 8 or depicted in Figure 9 is fashioned of a transparent material having an index of refraction close to that of water, such as a microporous polytetrafluoroethylene membrane, cells resting on bottom surface may be observed and studied optically from above or below vessel 10.

**EXPERIMENTAL**

A solution of approximately 18000 MOLT non-adherent cells in 50 microliter Dulbecco's phosphate buffered saline (PBS) were placed in each microwell of a standard 96-well plate (Nunc A/S, Roskilde, Denmark). After all the cells had settled  
5 (about 5 minutes) a current damper of the present invention substantially similar to current damper 94 depicted in Figure 10 was associated with the 96-well plate so that a damping component 86 was disposed within each one of the microwells of the 96-well plate. Each damping surface 26 was made of 3 micron microporous polycarbonate filter membrane (Osmonics, Inc, Minnetonka, MN, USA). The current damper was  
10 configured to suspend damping surfaces 26 200 microns above the microwell bottoms. The positions of cells resting on the bottom surface of a microwell were observed through the bottom surface of the microwell using an inverted microscope. Addition or removal of fluid into or from second volumes 34 did not cause any observable motion of the cells. 50 microliters of 4.8 micromolar Fluoresceine diacetate dye was added into  
15 second volume 34. The immediate emission of the characteristic Fluoresceine diacetate fluorescence from the cells indicated that the damping surface 26 did not cause a delay in delivery of the dye.

A current damper of the present invention substantially similar to current damper 94 depicted in Figure 10 was associated with standard 96-well plate (Nunc A/S,  
20 Roskilde, Denmark) so that a damping component 86 was disposed within each one of the microwells of the 96-well plate. Each damping surface 26 was made of 8 micron permeable collagen (Cellagen®, ICN Biomedicals Inc., Aurora, OH, USA). The current damper was configured to suspend damping surfaces 26 1 mm above the microwell bottoms. 50 microliters of a solution including approximately 20000 U937 non-adherent  
25 human promonocytes suspended in Dulbecco's phosphate buffered saline (PBS) were added through the tubular internal wall 40 of each damping component 86 and observed to form a single layer on the microwell bottom. The positions of cells resting on the microwell bottom surface were observed through the bottom surface using an inverted microscope. Addition or removal of fluid from second volume 34 did not cause any  
30 observable motion of the cells. Cells were removed from and added to the microwells using a pipette through a tubular internal wall 40.

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It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided  
5 separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art.

For example, although the embodiments depicted above are discussed with  
10 reference to a microwell of a 96-well plate with or without a picowell array on the bottom surface, the teachings of the present invention are applicable to any vessel used in the study of cells including but not limited to beakers, bottles, cups, flasks, plates, slides with recesses, tissue culture plates, vials, wells, microwells and Erlenmeyer flasks.

For example, although the embodiments depicted above include a single  
15 damping component disposed in a given vessel, the teachings of the present invention are also of a vessel having two or more damping components.

For example, although in the embodiments depicted above a vessel is divided  
20 into two volumes, a first volume including part of the bottom surface of the vessel and a second volume accessible from the surroundings and in fluid communication with the first volume through a damping component, the teachings of the present invention also include a vessel divided into more volumes, generally such volumes accessible from the surroundings and in fluid communication with the first volume through a damping component, analogously to the second volume.

For example, in the embodiments depicted above it is seen that various  
25 proportions of the area of an opening of a vessel 10 allow access to a respective first volume 36 and to a respective second volume 34 from surroundings 16. For example, in the embodiments depicted in Figure 2A or Figure 8 substantially all the area of the opening of vessel 10 allows access to second volume 34 from surroundings 16, while in  
30 the embodiment depicted in Figure 6 approximately 10% of the area of the opening of vessel 10 allows access to second volume 34 and 90% of the area allows access to first volume 36. In general, according to the teachings of the present invention any useful

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proportion of the area of an opening of a vessel 10 allows access to a respective first volume 36 and to a respective second volume 34 from surroundings 16. Generally at least 5%, at least 10% and even at least 20% of the area of the opening of a vessel 10 allows access to a respective second volume 34. In embodiments of the present invention a first volume 36 is substantially not accessible from the surroundings, although in embodiments of the present invention at least 5%, at least 10% and even at least 20% of the area of the opening of a vessel 10 allows access to a respective first volume 36.

Accordingly, the present invention is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In case of conflict, the specification herein, including definitions, will control. Citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

20

WHAT IS CLAIMED IS

1. A device for the study of cells, comprising:
  - (a) a vessel including a bottom surface and a wall having a bottom edge, a top edge defining a rim of said vessel, said rim surrounding an opening; and
  - (b) a current damper including a damping component substantially disposed within said vessel.
2. The device of claim 1, wherein said damping component substantially divides said vessel into at least two volumes, a first volume including at least part of said bottom surface and a second volume in fluid communication with the surroundings and with said first volume.
3. The device of claim 1, wherein said current damper is fixedly associated with said vessel.
4. The device of claim 1, wherein said current damper is discrete from said vessel.
5. The device of claim 1, wherein said damping component is discrete from said vessel.
6. The device of claim 1, wherein said damping component comprises a damping surface.
7. The device of claim 6, wherein said damping surface is substantially planar.
8. The device of claim 7, wherein said damping surface is substantially parallel to said bottom surface.

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9. The device of claim 6, wherein said damping surface is permeable to liquids.

10. The device of claim 9, wherein said damping surface is a net.

11. The device of claim 9, wherein said damping surface is a membrane.

12. The device of claim 11, wherein said damping surface is a microporous membrane.

13. The device of claim 6, wherein said damping component substantially divides said vessel into two volumes, a first volume including at least part of said bottom surface and a second volume in fluid communication with the surroundings and with said first volume.

14. The device of claim 13, wherein fluid communication between the surroundings and said first volume is substantially only via said second volume.

15. The device of claim 13, wherein said fluid communication between said first volume and said second volume is substantially only through said damping surface.

16. The device of claim 13, wherein said damping component is configured to optionally allow substantially unimpeded fluid communication between said first volume and the surroundings.

17. The device of claim 16, wherein said substantially unimpeded fluid communication is through a gap in said damping surface.

18. The device of claim 16, wherein said substantially unimpeded fluid communication is past a side of said damping surface.

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19. The device of claim 6, wherein said current damper is configured to allow at least part of said damping component to rest on the surface of a liquid contained in said vessel.

20. The device of claim 19, wherein said part of said damping component is configured to float on the surface of a liquid contained in said vessel.

21. The device of claim 19, wherein said vessel is substantially parallel-walled and wherein said damping component has an outer edge having dimensions substantially similar to those of a cross-section defined by said parallel walls.

22. The device of claim 6, wherein said damping component is configured to maintain said damping surface at a substantially fixed distance above said bottom surface.

23. The device of claim 22, wherein said distance is not greater than about 5 mm.

24. The device of claim 6, wherein said current damper further comprises a retaining component configured to physically engage said top edge of said vessel.

25. The device of claim 24, wherein said retaining component and said damping component are fixedly associated.

26. The device of claim 24, wherein said retaining component and said damping component are moveably associated.

27. The device of claim 6, wherein said current damper further comprises a retaining component configured to rest on said bottom surface of said vessel.

28. The device of claim 27, wherein said retaining component and said damping component are fixedly associated.

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29. The device of claim 27, wherein said retaining component and said damping component are reversibly associated.

30. The device of claim 6, wherein said current damper comprises a plurality of said damping components, each damping component configured to be disposed within a separate vessel.

31. The device of claim 30, wherein said plurality is selected from the group consisting of 6, 12, 24, 48, 96, 384 and 1536.

32. A method for the study of cells comprising:

- (a) providing a vessel including a bottom surface and a wall having a bottom edge, a top edge defining a rim of said vessel, said rim surrounding an opening;
- (b) placing at least one cell in a liquid at a location on said bottom surface;
- (c) disposing a damping component of a current damper within said vessel; and
- (d) adding a material to said vessel

wherein said damping component damps currents thereby reducing movement of said at least one cell from said location as a result of said adding of said material.

33. The method of claim 32, wherein said damping component substantially divides said vessel into at least two volumes, a first volume including at least part of said bottom surface and a second volume in fluid communication with the surroundings and with said first volume.

34. The method of claim 33, wherein said adding said material is into said second volume.

35. The method of claim 34, wherein subsequent to said adding, said material passes into said first volume.

36. The method of claim 32, wherein (b) precedes (c).

37. The method of claim 32, wherein (c) precedes (b).
38. The method of claim 32, said damping component comprising a damping surface and wherein said adding said material includes contacting said material with said damping surface.
39. The method of claim 38, wherein said damping surface is substantially planar.
40. The method of claim 39, wherein said damping surface is substantially parallel to said bottom surface.
41. The method of claim 39, wherein said damping surface is substantially unparallel to said bottom surface.
42. The method of claim 39, wherein said damping surface is substantially perpendicular to said bottom surface.
43. The method of claim 38, wherein said damping surface is substantially entirely submerged in said liquid.
44. The method of claim 40, wherein said damping surface is substantially at a fixed distance from said bottom surface.
45. The method of claim 44, wherein said distance is not greater than about 5 mm.
46. The method of claim 38, said damping surface is porous and wherein subsequent to said adding, said material passes through said porous damping surface.

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47. The method of claim 32, wherein at least part of said damping component rests on the surface of said liquid.

48. The method of claim 32, wherein at least part of said damping component rests on said bottom surface of said vessel.

49. The method of claim 32, wherein said damping component is suspended within said vessel above said bottom surface.

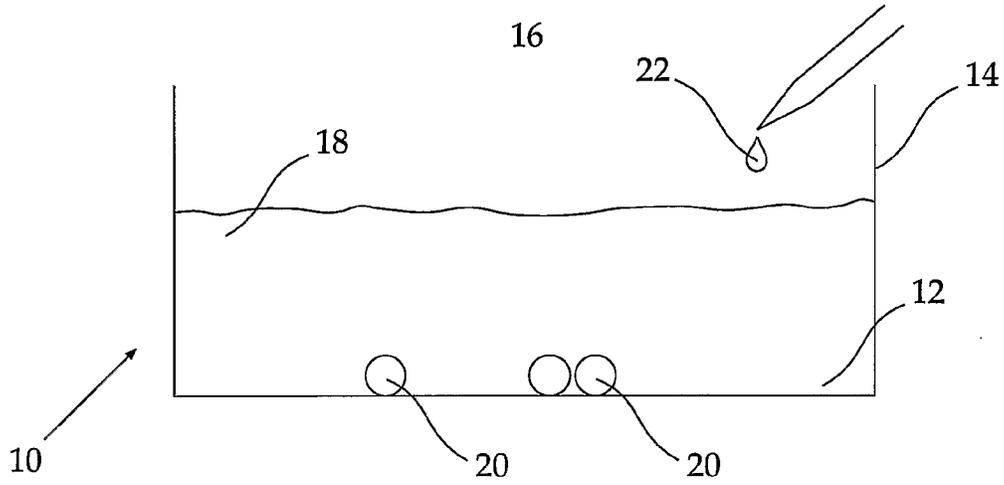


FIG. 1A (prior art)

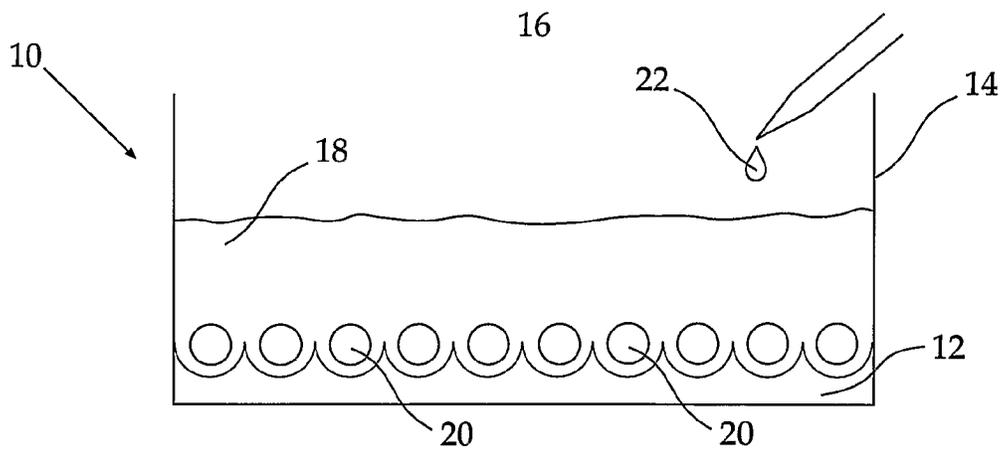


FIG. 1B (prior art)

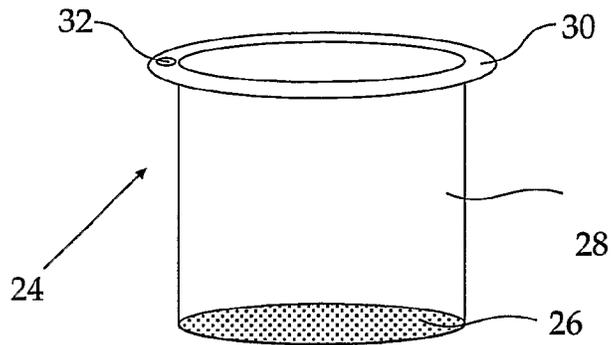


FIG. 2A

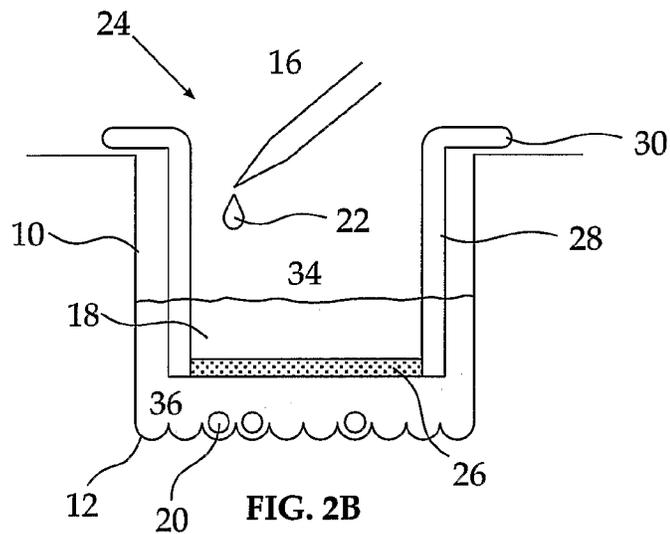


FIG. 2B

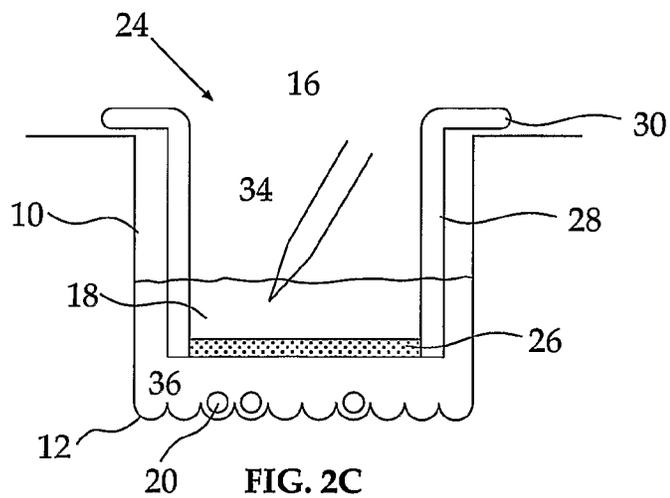
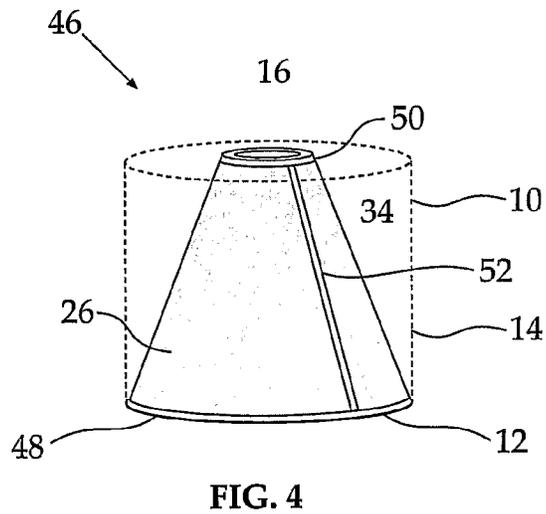
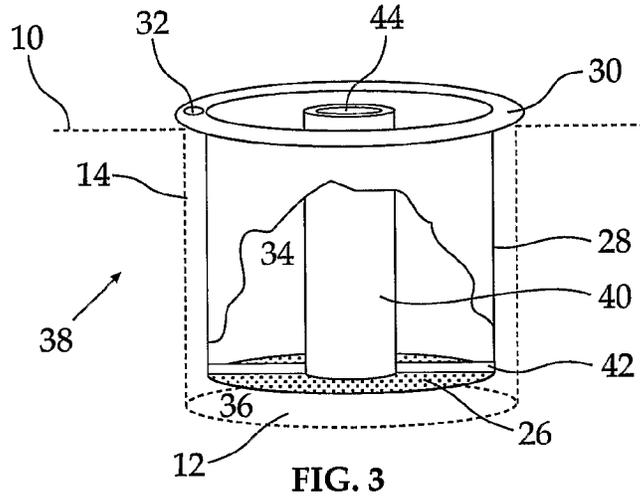


FIG. 2C



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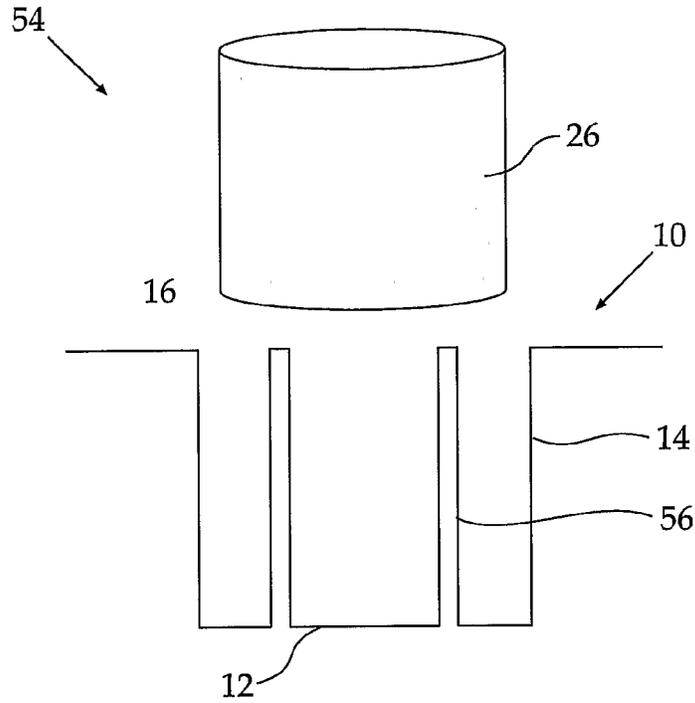


FIG. 5A

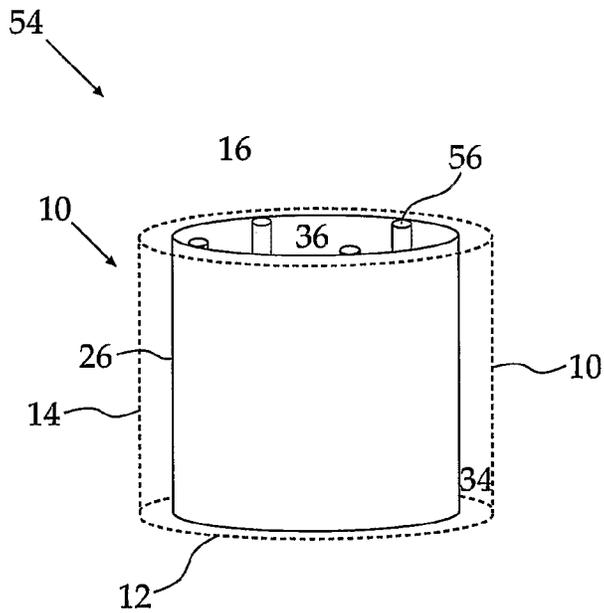


FIG. 5B

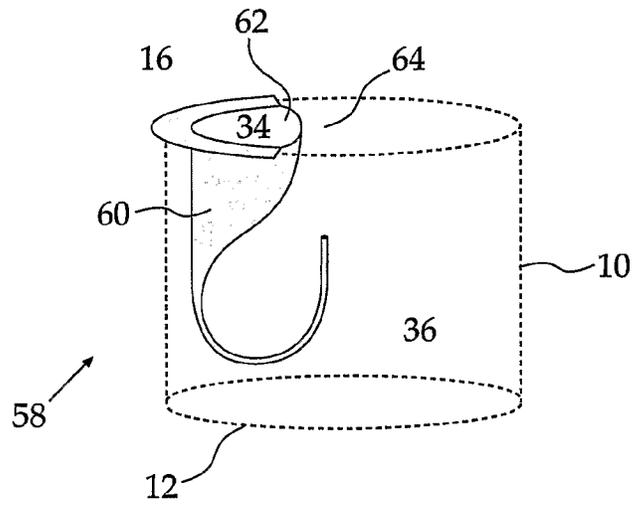


FIG. 6

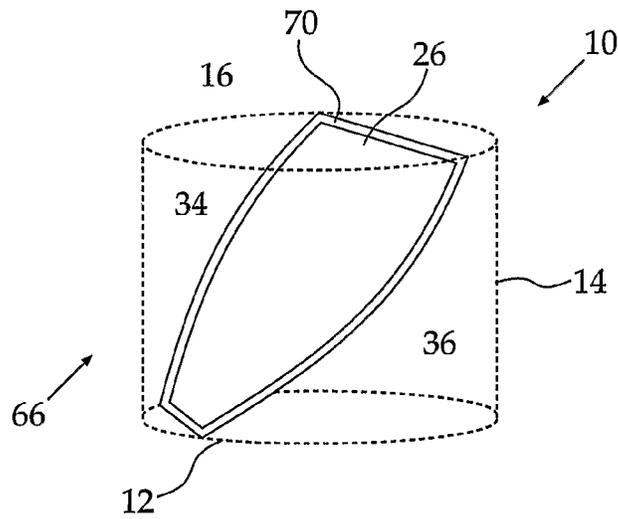


FIG. 7

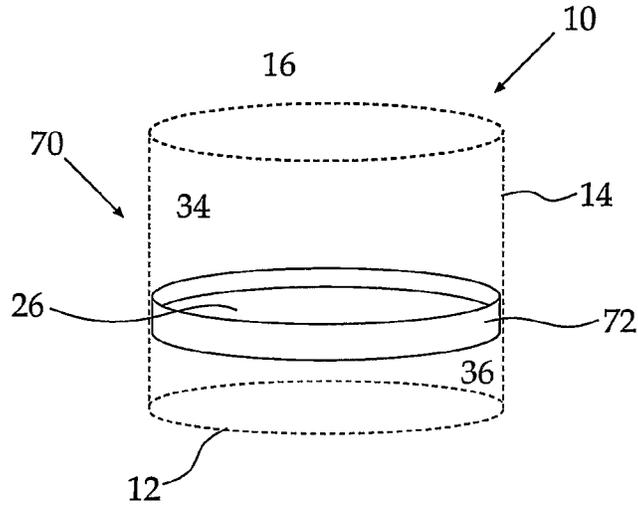


FIG. 8

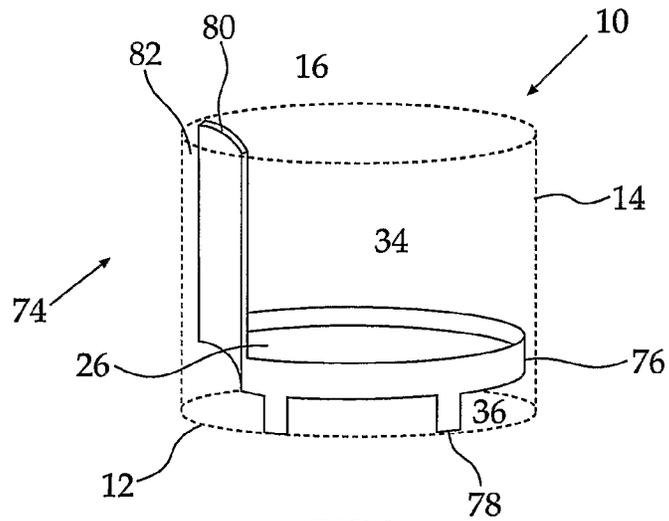


FIG. 9

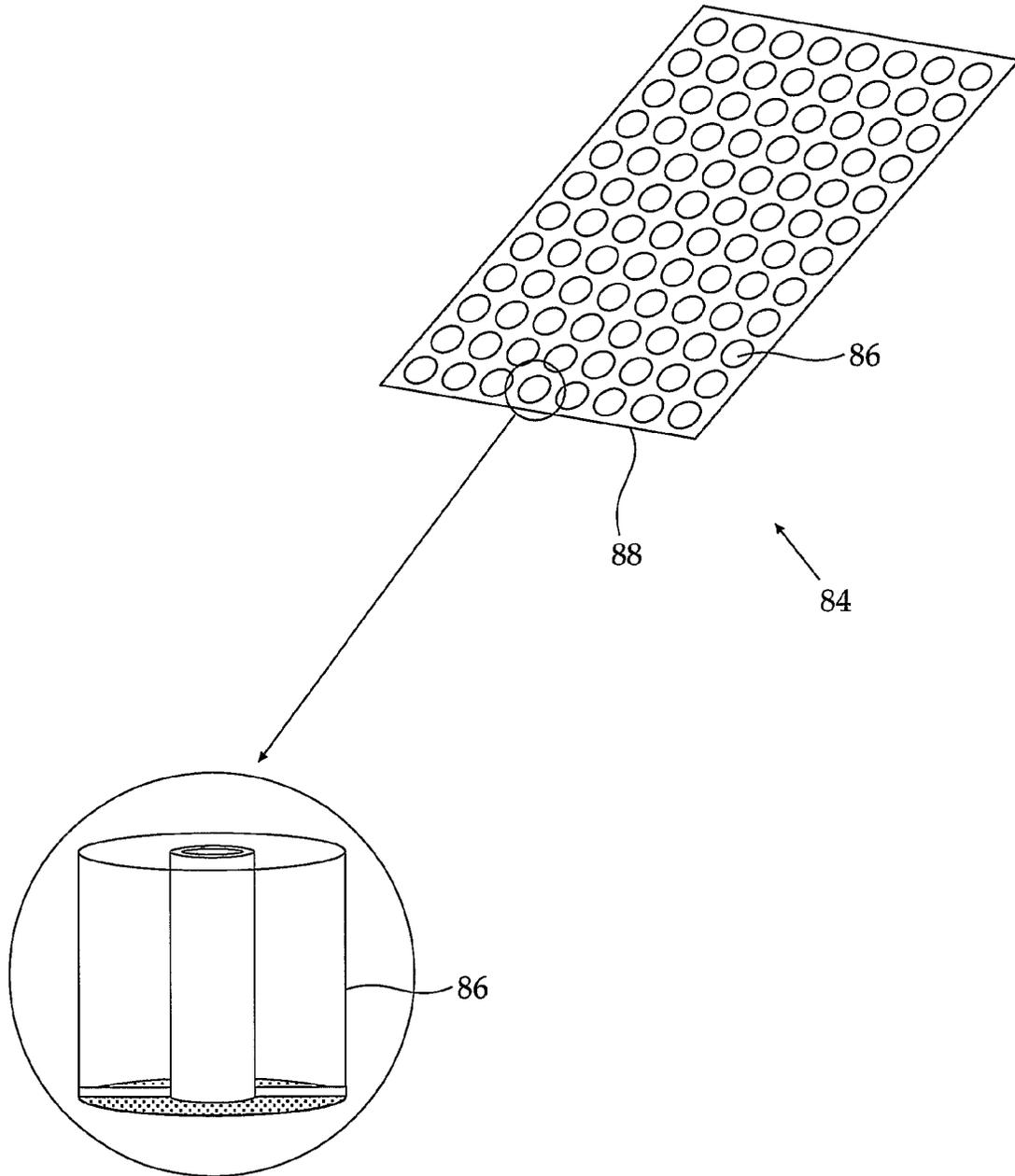


FIG. 10

# INTERNATIONAL SEARCH REPORT

Inte application No  
PCT/IL2005/001078

|   |  |   |
|---|--|---|
| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>G01N15/14      B01L3/00      C12M1/22   |  |   |
| According to International Patent Classification (IPC) or to both national classification and IPC   |  |   |
| <b>B. FIELDS SEARCHED</b>   |  |   |
| Minimum documentation searched (classification system followed by classification symbols)<br>G01N    B01L    C12M   |  |   |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched   |  |   |
| Electronic data base consulted during the International search (name of data base and, where practical, search terms used)<br>EPO-Internal , PAJ  |  |   |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>   |  |   |
| Category*   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No                    |
| X   | US 5 707 869 A (WOLF ET AL)<br>13 January 1998 (1998-01-13)<br><br>column 2, line 37 - column 3, line 33<br>column 5, line 14 - column 6, line 11<br>column 9, line 7 - column 10, line 46<br>-----  | 1-16,<br>19-24,<br>27, 28,<br>32-47, 49 |
| X   | US 2002/189374 A1 (DESILETS KENNETH ET AL)<br>19 December 2002 (2002-12-19)<br>paragraphs '0032! - '0038!; figures 1-4<br>-----  | 1-31                                    |
| X   | US 2003/124716 A1 (HESS ROBERT A ET AL)<br>3 July 2003 (2003-07-03)<br>paragraph '0201!; figure 23<br>-----<br>-/---   | 1-3,<br>16-18                           |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex   |  |   |
| * Special categories of cited documents   |  |   |
| "A" document defining the general state of the art which is not considered to be of particular relevance<br>"E" earlier document but published on or after the international filing date<br>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art<br>"B" document member of the same patent family |   |
| Date of the actual completion of the international search<br><br><p style="text-align: center;">31 January 2006</p>   | Date of mailing of the international search report<br><br><p style="text-align: center;">07/02/2006</p>  |   |
| Name and mailing address of the ISA/<br>European Patent Office, P B 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel (+31-70) 340-2040, Tx 31 651 epo nl,<br>Fax (+31-70) 340-3016   | Authorized officer<br><br><p style="text-align: center;">Tiede, R</p>  |   |

# INTERNATIONAL SEARCH REPORT

Inter application No  
PCT/IL2005/001078

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |  |   |
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| Category*  | Citation of document with indication where appropriate, of the relevant passages   | Relevant to claim No                          |
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